We produced MBP-tagged and biotinylated peptides for studying the interaction between aldolase and TRAP using surface plasmon resonance. We have screened the Medicines for Malaria Ventures library and have identified several compounds with concentration-dependent effects *in vitro*. Five promising compounds were moved forward into parasite gliding and invasion assays. We have observed phenotypic differences in the ability of *Plasmodium* parasites to glide, and found a reduced gliding velocity and abnormal rates of sporozoite attachment and detachment. To determine the effect on invasion, both red blood cell and hepatocyte invasion assays have been performed. We are currently attempting to determine a co-crystal structure of the ternary aldolase-TRAP-compound complex, and to move promising compounds into an *in vivo* mouse model.

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Absolute Binding Free Energy Calculations of Bromodomain Inhibitors

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Computational approaches have been increasingly employed for drug discovery in both academia and industry. The ultimate goal of rational drug design is the prediction of the biological activity of a compound, and such activity is driven, at the molecular level, by the specific intermolecular interactions between the small organic molecule, the biological target and the solvent. Similar molecular recognition processes are crucial for many biological functions and the strength of the recognition is characterized by its binding affinity. Thanks to important advances in theory and computing in the last decades, predictions of binding affinities using physics-based simulations are gaining popularity. In particular, binding free energy estimates based on alchemical pathways have been shown to be a rigorous approach for the affinity prediction problem and hold the promise to be able to guide lead optimisation. However, whilst relative calculations have made significant contributions in a drug-discovery context, absolute calculations have so far been mostly applied to model systems despite the advantages related to the ability to estimate affinities for largely diverse sets of molecules.

We present the results of a study where we evaluated the performance of alchemical free energy estimates for a set of drug-like molecules that have been developed to target bromodomains, a family of epigenetic marks readers with established therapeutic potential. The study evaluates the performance of the protocol employed from a retrospective and perspective standpoint, with particular attention to the precision of the calculations and comparing the theoretical results with high-quality binding data. We show that, for this epigenetic target, good agreement between calculations and experiments is achievable even for very challenging compounds, suggesting that alchemical free energy calculations might be approaching the degree of reliability required in order to have an impact in drug discovery campaigns.

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Positive Modulators of Glycine Receptors with Analgesic Potential Identified by Virtual Screening

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Human glycine receptors (hGlyRs) are important targets for neuroactive drugs, including for analgesic therapies. A previous study has identified the crucial role of residue S296 in hGlyR-α1 potentiation by Δ9-tetrahydrocannabinol (THC) and in cannabinoid-induced analgesia. The recently determined NMR structure of the hGlyR-α1 transmembrane domain (TMD) provides a basis for structure-based virtual screening to discover new analgesic drugs. Using a large ensemble of hGlyR-α1 structures generated from molecular dynamics simulations based on the NMR structure, we screened 1549 FDA approved compounds from the DrugBank database targeted to a cannabinoid binding site near residue S296. Drugs were ranked based on their predicted binding affinities across the ensemble of hGlyR-α1 TMD structures. Four leading compounds were selected for experimental validation in Xenopus laevis oocytes expressing hGlyR-α1. At a low concentration (1 μM), all four leading compounds potentiate hGlyR-α1 currents more than two times, which was greater than that of THC. The hit rate is remarkable. The study provides strong evidence that these leading compounds will be at least as effective as THC for analgesia by acting on hGlyR-α1, but without psychoactive effects. The protocol developed here can easily be applied to the discovery of novel analgesic compounds of even higher efficacy on hGlyRs. Research supported by grants from the NIH and XSEDE.

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Green and Black Tea Polyphenols Mechanistically Inhibit the Aggregation of Amyloid-β in Alzheimer's Disease

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Alzheimer's disease (AD) is the 6th leading cause of death and is the only disease among the top 10 that cannot be prevented, cured or treated. The amyloid cascade hypothesis states that naturally occurring amyloid-β (Aβ) monomer aggregates via a nucleation-dependent pathway to form soluble aggregates and insoluble fibrils that deposit as plaques in the brain. Consequently, inhibition of Aβ aggregation is one therapeutic strategy for AD. This study sought to explain epidemiological correlations between frequent tea consumption and reduced incidence of AD by identifying the Aβ aggregation inhibitory capabilities of key polyphenol components in green and black tea.

Polyphenols studied include green tea catechins, epicatechin, epigallocatechin and epigallocatechin gallate, as well as black tea theaflavins, theaflavin and theaflavin monogallate. Four assays were used to target unique steps along the aggregation pathway: a monomer aggregation assay to monitor the overall aggregation process; an oligomerization assay to monitor the initial nucleation step; an association assay to monitor the late stage lateral binding of soluble aggregates; and an elongation assay to monitor the late stage lengthening of soluble aggregates.

Catechins and theaflavins show different inhibitory capabilities at varying mechanistic steps of the Aβ aggregation pathway. Catechins affect only the later stages of aggregation, suggesting that catechins may bind a specific structure present in aggregates. Conversely, theaflavins show inhibitory capabilities at every stage of aggregation, alluding to a sequence specific recognition. Furthermore, better inhibitory capabilities, for both polyphenol categories, can be correlated with the number of gallate groups.

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Effect of Reactive Aldehydes on Ionophore-Mediated Transmembrane Translocations of H^+ and K^+

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Reactive aldehydes (RA) are involved in the onset and progression of many pathologies such as cardiovascular and neurodegenerative diseases. The precise mechanisms by which aldehydes contribute to these diseases remain unclear. Previously, we and other groups suggested that RAs modify the membrane protein function by either binding to the proteins directly(1) or to aminophosphosholipids, with a subsequent alteration in protein conformation. Here, we investigated the influence of biologically important RAs such as 4-hydroxy-2-nonenal (HNE), 4-oxo-2-nonenal (ONE) and 4-hydroxy-2-hexenal (HHE) on the transport kinetics of the ionophores carbonyl-cyanide-m-chlorophenylhydrazone (CCCP) and valinomycin (Val). We found that RA increases the membrane conductance, G, in the presence of Val in the following order ONE>HNE>HHE. In contrast G decreases in the presence of CCCP and ONE. The presence of phosphatidylethanolamine in the membrane was crucial for the effect to occur. The results are consistent with the hypothesis that RA adducts alter membrane boundary potential.

1. E. A. Malingriaux, et al., "Fatty Acids are Key in 4-Hydroxy-2-Nonenal-Mediated Activation of Uncoupling Proteins 1 and 2" PLoS. ONE. 8(10), e77786 (2013).

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Developing High-Throughput Fluorescence-Based Assays for Measuring Kinase Inhibitor Free Energies of Binding

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Understanding the specificity of kinase inhibitors has tremendous therapeutic significance. To predict inhibitor selectivity computationally, an iterative approach incorporating experimental measurements is ideal. This work describes the development of a high-throughput label-free fluorescent ligand-binding assay to measure inhibitor affinities. Taking advantage of the intrinsic fluorescence increase of a group of FDA-approved kinase inhibitors upon binding kinases, we are able to measure inhibitor binding affinity with small amounts of protein and without any fluorescent labels. This facilitates rapid characterization of a wide range of kinase inhibitors and kinase resistance mutants, within a system that can be reproduced identically in molecular dynamics simulations.